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ETIOLOGY OF TSUTSUGAMUSHI DISEASE *

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An endemic infectious disease, called Tsutsugamushi disease (Kedani fever, river fever), occurs in Japan along the rivers in the northern provinces. In spite of the persistent efforts of numerous workers, its etiology has been very difficult to determine. This peculiar disease has a close resemblance to Rocky Mountain spotted fever in America, but it is transmitted by a different insect. The carrier of the Japanese disease is a minute red mite, abundant in the infested region, that is, in the low lying cultivated fields along the Shinano river, between the riverside and embankments which vary up to one and a half miles from the river. These fields are at times during the summer covered by overflow water. In nature the mite is ectoparasitic in the ear of field mice, but it freely attacks human beings as well as other animals whenever accessible. Zoölogically, it is considered *Leptus akamushi* (Brumpt), a species distinct from the well known *L. autumnalis* of Europe.

The early clinical course of the disease is marked by a gradual rise of the body temperature, a characteristic lesion at the site of the bite, and swelling of adjacent lymph nodes. The incubation period is about eight days. The fever, which is usually 38°-39° C. in the early stage, may rise to 40° C. or even higher, and usually lasts three or four weeks. Complete recovery requires a month or more. In bad cases a fatal result follows about ten days after the onset. The mortality of the disease is approximately forty per cent.

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* My work on this disease was started in 1906 and has been continued up to 1919. Many of my findings in the course of these twelve years' investigation have been published in Japanese medical journals (Hokuetsu Igakkai Zasshi, no. 156, 1906; no. 158, 1907; no. 162, 1908; no. 165, 1909; no. 168, 1909; no. 173, 1910. Chu-o Igakkai Zasshi, no. 124, 1915; no. 127, 1916; no. 130, 1916. Hayashi, N., Mukoyama, T., Oshima, F.: Chu-o Igakkai Zasshi, no. 138, 1918; 1919 [XXVI] no. 2). A summary of these findings together with new facts and conclusions with regard to the etiology of this disease is of especial interest in connection with recent American contributions to the study of Rocky Mountain spotted fever. I have been very much pleased to find in Dr. Wolbach's recent detailed description of the organism found in the American fever, a very close similarity to the bodies which I found in the closely related Japanese disease.

The microscopical examination of fresh material in the hanging drop shows a number of refractile, spherical bodies, each measuring from 1 to 3μ in diameter, without any perceptible movement. They rarely occur in clusters, but two or three of them may often be seen linked together. As a rule, when two bodies are coupled, one is decidedly larger than the other. The smaller half of the couple often appears as if it were a pseudopodium of the larger. I discovered these peculiar bodies in 1906, and designated them as the Spheroid Bodies. The examination of blood shows, in addition to these, others which I have called the "rod-bodies." There are two kinds, the first large and the second very small; the large one measures about 7 by 3μ. It contains a spherical refractile area resembling a nucleus and a number of much more refractile granules toward the periphery, and is always found close to the surface of the red blood cells. The small "rod body" measures about 3 by 0.5μ and resembles a bacterium. It shows no evidence of motility.

Varying numbers of the spheroid as well as rod bodies are often found embedded in the cytoplasm of the cells of the exudate and of lymph nodes. Fresh sections examined in normal salt solution, glycerine or dilute acetic acid show these bodies intracellularly, and their morphological characters are identical with those that are found extracellularly.

The lesion which develops at the location of the bite is quite unique, being comparable only with the chancre of syphilis. In the earliest stage that can be identified, the wound is much raised and is about the size of a small pea, being 2-3 mm. in diameter. It has first a reddish purple color, and later a blackish necrotic appearance. A smear from the wound at this stage shows a large number of very small rod and spheroid bodies, but cultures taken from the same source at the same time are entirely negative. The wound usually commences to heal through the regeneration of surrounding tissues when the case is on the way to recovery, and thus shows close parallelism with the general clinical course of the disease. Obvious as is the importance of the study of the wound, it has received little attention. On account of its being exposed, thus offering opportunity for various secondary infections, the result of observations are of little value unless controlled by bacterial cultures in every case.

At the onset of the fever, the minute bodies appear in great abundance in the smear. Most of them are seen to be more or less enlarged. Even at this stage cultures do not demonstrate any bacterial infection. At the height of the fever, when the superficial, degenerated tissue of the lesions falls off, leaving a small ulcer, there usually occurs a secondary infection with common bacteria such as staphylococci, as

detected by cultures. In mild cases, the rod and spheroid bodies, as well as bacteria, gradually decrease in number, hand in hand with the decline in fever.

In later stages, with necrosis and ulceration of the lesion, the exudate usually contains bacteria. The wandering cells which appear in large number in the lesion are actively phagocytic. Some of these phagocytes contain in addition to degenerative products of red blood cells and tissue cells, a large number of rod as well as spheroid bodies. The manner in which the minute bodies are included in cytoplasm of these cells is identical with the conditions in the cells of lymph nodes.

The rod-bodies are extremely small, measuring 1-2, rarely 3μ in length. Loeffler's methylene blue and ordinary Giemsa solution stain them blue throughout like bacteria, but Prowazek's trachoma granule stain brings out in the rod-body, a circular area of chromatin substance staining purplish, leaving the rest of the body blue although not as distinct as in protozoa. Hematoxylin also gives a staining reaction of the circular area characteristic of chromatin. The rod-body is typically more or less pointed at one end, and slightly rounded at the other; the chromatin area being at the round end (Pl. X, Figs. 18, 24). Some of the rod-bodies show a chromatin area at each end (Figs. 19, 20, 21). In those that are as long as 3μ the chromatin substance may appear to lie outside the body and attached to one end of the latter by a narrow belt of achromatic substance. (Pl. X, figs. 25, 26; Pl. XI, figs. 3, 5). The largest of the rod-bodies may measure 7 by 2μ and may even at a glance appear like trypanosomes (Figs. 15, 16). These giant forms also contain either at the end or middle, one or two masses of chromatic substance.

These spheroid bodies are comparatively large, being $2\text{-}3\mu$ in diameter, and stain deeply like cocci. A characteristic figure often seen shows two spheroid bodies of distinctly different sizes coupled together (Pl. X, Figs. 27, 28, 31), or in a manner resembling diplococci (Fig. 29, 30, 6 B), or even in chain formation. Frequently chromatic substance in the center stains more deeply than the peripheral area. These characteristic features are more conspicuous in larger forms than in smaller ones.

The swelling of lymph nodes adjacent to the bite takes place in the early clinical stage, when the wound is still elevated and the body temperature only slightly raised. Although some lymph nodes were removed by operation in an early stage the greater part of my material was obtained after the development of distinct fever (38.5°-39° C.). All of the lymph nodes in the region manifest swelling though in different degree. At the height of the fever, material was often taken by puncture with the syringe; it was collected at autopsy, and also by operation at various stages in experimental infection of animals.

Examination of sections and smear preparations has shown the presence of peculiar granules, which are taken up by the giant phagocytes of endothelial nature. These granules and phagocytes occur most abundantly around the bite in the adjacent lymph nodes and spleen. Morphologically, three types of granules and phagocytes are found in the cytoplasm of the large mononuclear lymphoid cell, namely:

(a) A large ring-shaped chromatic body with an achromatic area (Pl. I, 1a; Pl. XI, Figs. 10a, 11a, 12a, 13a).

(b) A spherical body showing deep and uniform blue staining, but containing reddish chromatin substance having a bipolar distribution, and resembling a diplococcus (Pl. IX, Fig. 1B, 1b).

(c) An exceedingly small rod shaped body, often more of comma-shape, or with dumb-bell-like construction. This third type of the granules is most abundant (Pl. IX, Figs. 2, 3, 4, 5).

In 1915, I found granules identical with those just described in small lymphocytes from lymph nodes removed early by operation. Examined with the Giemsa-Prowazek stain, minute granules can be demonstrated in the narrow area of blue cytoplasm around the purple nucleus. With the aid of a Zeiss $\frac{1}{12}$ objective and ocular 6 or 8, the granules can be easily classified into the three types already described. Here, more than in other cases, the rod-shaped type predominated. The rod-body is more or less rounded at one end, and pointed at the other. A proper stain shows the round end reddish purple, and the pointed end blue. Delafield's hematoxylin brings out similarly the three types of granules, and also differentiates clearly the round end of the rod-body.

In general, the granules are fairly constant in size. The number embedded in a single cell varies from a few to approximately two hundred, but more commonly from ten to sixty. These are usually more or less localized in the enlarged portion of the cytoplasm (Pl. IX, Fig. 2; Pl. XI, Figs. 2a, 3a), or in the area of the cytoplasm formed by the indented nucleus.

The lymph (from lymph nodes) also contains three types of the granules. Here, too, the granules may be found in clusters in many cases, although some may be solitary. In addition, the fluid often contains a fourth type of granule, round in shape with a centrally located red-staining spot and a narrow achromatic ring, which is in turn surrounded by a finely granular peripheral zone (Pl. XI, Figs. 17, 18, 19). I have given the name of "oocystoid body" to this type.

It is natural to suppose that the virus of Tsutsugamushi disease may be found in the blood, after it enters the body of the patient through the bite. Its demonstration would be of great value, especially as affording a comparatively simple means of accurate diagnosis. Cover

glass smears from a large number of severe cases all showed the presence of the peculiar granular bodies, in or on the red corpuscles. In mild cases, or those in very early or late stages, none, or a very small number of the bodies were observed. These bodies may also occur in any type of white blood cell, and, moreover, may be seen scattered freely in the blood plasma. These facts I reported in 1906.

The granular bodies are minute, strongly refractile, and usually found within or on red corpuscles (Pl. X, Figs. 6c, 7). A highly magnified picture of Giemsa-Prowazek stained specimens (Pl. X, Figs. 6, 7) shows that these bodies are in no way different, as far as could be observed, from the minute bodies already described in the lymphoid cells. They are usually comma-shaped, but often modified into rod or dumb-bell shapes. They are, as a rule, evenly distributed within the cell, although sometimes grouped in one part of it. The number of the bodies in a red cell varies from a few to many. Enlarged forms, such as are found in the cells of the lymph nodes, also occur in the blood (Pl. X, Fig. 6A, 14). The minute bodies may be seen free in the blood plasma during the eruptive stage even in the case of a slight attack. The smaller forms of these extra-cellular bodies may appear in a thickly crowded group (Pl. XI, Fig. 1, 16a), while some of the larger forms may be found coupled with smaller ones (Pl. X, Figs. 15, 16, 17). This phenomenon of coupling among the larger bodies is of special interest, as it takes place only in very severe cases.

Sometimes with the Giemsa-Prowazek stain a chromatin mass surrounded by an achromatic ring was seen at one end of the body (Pl. X, Figs. 12, 13, 14). Some bodies are round (Figs. 12 13), others elongated (Fig. 14). The former is like a ring body of the malaria plasmodium; the latter has two (end and center) chromatin spots, the central being very small. Delafield's hematoxylin also brings out these chromatin spots.

The enlargement of the spleen in Tsutsugamushi disease, usually encountered at autopsy, can be easily detected clinically. Material taken by puncture with a syringe always contains a large quantity of red cells, but pulp-cells are rarely seen. The minute granular bodies are abundantly demonstrated in these cells in many cases. This is also true in the case of autopsy material. Generally speaking, the smaller type of granular bodies seems to predominate in this organ (Pl. XI, Fig. 8a).

ANIMAL EXPERIMENTS

1. Infection through the bite of the mite: Animals (monkeys, rabbits, guinea-pigs, rats and calves) were allowed to be attacked by the mites in the infested region, and after recognizing the evidence of the bite on them, development of symptoms was carefully watched for.

The monkey shows the most typical rise of body temperature, the guinea-pig a less typical fever, and the calf only a slight rise of temperature. The rabbit shows no change.

Positive infection of a monkey (*Pithecus fuscatus* Blyth, the short-tailed species occurring in Thikoku, Japan) through the bite of the mite was first proved by the author in 1906. In this animal, the typical lesion of the bite developed, along with the enlargement of the lymph nodes and the characteristic fever, four or five days after the bite was recognized. The general clinical changes closely resemble those in human cases, even to the occurrence of leukopenia. The characteristic minute bodies, described from human cases, are present in the phagocytic cells of lymph nodes and spleen; in a later stage, also in those of bone marrow, and still later in most of the other internal organs. These bodies were also found free in body fluids. The infected monkeys usually recover in a week or two.

Guinea-pigs show no special pathologic change at the site of the bite, although swelling of adjacent lymph nodes and fever develop within several days after they are bitten. In these cases, too, the minute bodies of several types were abundantly demonstrated in the lymph nodes, blood and other tissues.

2. Transmission Through Injection of Infected Blood: The typical symptoms of the disease were successfully reproduced in the monkey by subcutaneous injections of the blood from the severe human cases. The diseases can also be transmitted from the monkey thus infected to other monkeys by inoculating them with blood drawn from the former. Similar experiments with guinea-pigs also gave positive results. Moreover, the injection of blood or emulsified organs from infected guinea-pigs into other animals, into monkeys, was followed by the development of unmistakable symptoms.

3. The Relation of the Mite and Field Mice: As stated already, the mite is parasitic on the inside of the external ear of the field mouse, which is very abundant in the infested region. In 1910, I was able to demonstrate that the field mouse is a bearer of the virus of Tsutsugamushi disease.

Aerobic and anaerobic cultures, on various solid as well as liquid media, were repeatedly taken from a large number of human and animal cases, always with negative results. A few of the common varieties of bacteria or often yeasts developed in some cultures, but no organism that can be considered as the causative agent of the disease has ever been detected in any of them.

By using Kleine's (1905) piroplasm medium, it was possible to recognize the presence of the minute bodies described in this paper, but no definite growth has been demonstrated on this or on the Novy-Mac-

Neal-Nicoll medium. The cocci bodies, grown on Loeffler's serum-agar by Nagayo and his associates in 1917, could not be found in repeated experiments which I made with the same medium. The possibility of cultivating the virus of the disease has not thus far been definitely demonstrated.

Under natural conditions healthy individuals do not contract the infection directly from patients suffering from the disease, the virus being transmitted only through the mite. It has been noted in earlier publications that a certain degree of immunity follows recovery from this disease. A second infection may occur some years later but the case is never severe. A third, or even fourth infection, in a very light form is also known. In animals, however, I found that no second infection was possible, in spite of many trials. I have also noted that the first infection is very mild in children or in young individuals, while in adults over forty it is frequently fatal.

The resistance of the virus to thermal change and putrefaction is very slight, as virus from human as well as animal cases is no longer virulent a day or two after it has been taken. Another important fact is that the virus is absent from the filtrate through Pukall or Berkefeld filters. This point, first reported by Kitajima and Miyajima, was recently confirmed by Nagayo.

DISCUSSION

1. The Granular Bodies in the Lymphoid Cells: The larger types of these ring or spheroid bodies are found in large mononuclear cells, as well as in the cells of the spleen pulp, while the minute type (rod bodies) are embedded in the cytoplasm of small mononuclear lymphoid cells.

Since Ehrlich's classical work it has been believed until very recently that the mononuclear lymphoid cell is not granulated under either normal or pathological conditions. Among others, Pappenheim and Schridde have shown the occurrence of fuchsinophile granules, which are now known to be the true mitochondria. More recently azure granules of problematic nature have also been described in this type of cells. These granules are, however, of different chemical nature from my granular bodies, as can be seen from their staining reactions. It is beyond doubt that the former could not be considered identical, or even genetically related to the latter. An extensive examination has failed to demonstrate the granular bodies in lymphoid cells in normal individuals, leading to the conclusion that they are not normal constituents of these cells. Nor have the granular bodies been observed in the lymphoid cells in any case of lymphoid hyperplasia that I have examined.

The impossibility of the granular bodies being any sort of degeneration products, which might become phagocyted by lymphoid cells, is none the less evident in view of my microscopical observations. The morphological characters and the regularity in the modification of these bodies, as detailed early in this paper, preclude any conclusion that they are degenerative debris, and suggest very strongly their being peculiar micro-organisms. For the same reasons the granular bodies must be considered as different from Prowazek's so-called "reaction products" in the infections of various sorts of filtrable virus. The granular bodies in question are absolutely peculiar to Tsutsugamushi disease and must not be overlooked in the study of its etiology.

2. The Granular Bodies in the Large Mononuclear Cell: The large mononuclear cell, called endothelial in my report in 1916, Kiyono terms histiocyte. The occurrence of the granular bodies in this type of cells is a common finding in Tsutsugamushi disease. The granules are either rod-shaped, spherical or ring-shaped, and these three types may be found side by side in a single cell. They are identical in staining reactions but differ in size. It is very probable that the three types represent stages in a series of modifications undergone by the same organism. It seems that the minute rod-shaped body gradually grows, assumes a spherical form and then after further growth the ring shape. The semilunar, or crescent shape often taken by some much enlarged bodies may be considered as due to still further growth. Some of the ring-shaped bodies may also be coupled. It is very interesting to find on close examination that these enlarged bodies contain a number of exceedingly minute granules. If the interpretation suggested proves to be correct, the granular body must be an organism, and more specifically a protozoon, which has intracellular stages in its life cycle.

3. Granular Bodies in the Red Cell: In view of the fact that these bodies resemble the ordinary basophilic granules in the red cells, the distinctive characteristic between them may be pointed out. As first made known by M. Askanazy, Grawitz and others, the basophilic granules of red cells appearing under certain pathologic conditions, are nothing more than ill-defined, minute particles. Contrary to this the "granular bodies" found by me are large enough to be measured. Moreover, they are very clearly differentiated from the protoplasm of the red cells. On destaining the true basic granules become obscure very rapidly, while the "granular bodies" stand out clearly by their peculiar refractility, sharply defined edges, and finally by the characteristic chromatin spots which still retain a reddish purple color.

In short, the "granular bodies" with their organized structures cannot be confused with the true basic granules which have no definite

morphological characteristics. I conclude, therefore, that the "granular bodies" in the red blood cell are of the same nature as those in the lymphoid cells. Since the red cells as well as the lymphoid cells contain the "granular bodies" only in cases of Tsutsugamushi disease, these bodies must be considered seriously in connection with the etiology of the disease.

4. Free Bodies in Plasma: In the foregoing I have stated that the minute bodies usually found embedded in the cytoplasm of lymph cells may also occur free in the lymph, either solitary or in groups, and that these bodies may be rod, spheroid, or ring-shaped. An oocyst-like appearance was also assumed by some of them. Swarms of the minute "comma" or "rod-bodies" often mixed with longer dumb-bell shaped ones, were observed in the plasma in blood smears. Also these bodies have been found in addition to bacteria in serum from the wound at the site of the bite.

The free bodies are not abundant in mild cases but in severe cases their appearance is conspicuous. The free bodies often appear earlier than the intracellular bodies, and may occur in the wound along with ordinary bacteria.

Since the minute bodies in lymphoid cells are peculiar to Tsutsugamushi disease, the free bodies should also be considered of the same significance inasmuch as they have not been found under any other conditions. Morphologically, moreover, a minute comparison fails to detect any perceptible difference between the intracellular and free forms.

In their staining reactions and morphology alone the bodies which I have described often resemble bacteria, especially as the cytoplasm and chromatin substance are differentiated with difficulty, but there is no other evidence of their bacterial nature. In smears from blood and lymph nodes I have found bodies in both red cells and lymphocytes identical in appearance with the ring forms found in the red cells in malaria (Pl. IX, Fig. 1a; Pl. XI, Figs. 4b, 5b), showing a structure never found in bacteria.

I described these minute granular bodies in my first report on the virus of Tsutsugamushi disease and from various findings concluded that they represent a species of protozoa. It was suggested that the organism might be either Piroplasma or some related form. In a further paper I classified the organism as Piroplasma, referring to *Theileria parva* of African cattle fever, and tropical pirosomes in cattle described by Dschunkowsky and Ruhs, as most closely related forms.

In order to indicate the basis of my conclusion, the following facts should be emphasized :

1. Tsutsugamushi disease is transmitted by certain species of mite; the original bearer of virus in the infected region is the field mouse on which the mite is parasitic.

2. Tsutsugamushi disease is characterized clinically by the lesion, high fever, and swelling of lymph nodes. Decrease in the number of leukocytes is well marked in the blood picture. There is also a gradual decrease in red cells, but it is not conspicuous.

3. Anatomically the lesion is characteristic, also the swelling of lymph glands and splenic enlargement; the blood is less coagulable. Internal organs undergo parenchymatous degeneration and often show small areas of necrosis and thrombi.

4. Experimentally it is possible to transmit the infection with typical symptoms by inoculation of fresh blood or other tissues, from human as well as animal cases experimentally.

5. No bacteria which can be considered as the cause of the disease have been cultivated on media, either from clinical or experimental material, nor have any spirochaetes been found.

6. Under normal conditions the virus is not transmitted directly from man to man. It is least resistant to dryness and heat, and unfilterable.

With the above facts on one hand, and on the other the knowledge that the rod, spheroid and ring-shaped bodies, occur in all internal organs and in the blood and especially because of the early appearance of the rod-bodies in lymph cells adjacent to the bite, I have reached the conclusion that the virus of the disease is the species of Piroplasma in question. Among various species of Piroplasma, the cause of African cattle fever, *Thieleria parva,* (see Gonder, 1911), seems closely allied to the forms found in Tsutsugamushi disease, on account of morphological similarity, and of affinities for lymphocytes or endothelial cells.

In the case of *Theileria parva,* however, it is impossible to infect normal cattle by subcutaneous injection of diseased blood, while in Tsutsugamushi disease such a transmission is easily secured through an injection of a very small quantity of infected blood. In this respect the tropical pirosomes, described by Dschunkowsky and Ruhs (1904) from the Caucasus, shows a closer approach to my organism since in the former infection can be transmitted mechanically, as in the latter. In addition the tropical pirosome agrees with my granular bodies of Tsutsugamushi disease in having an intracellular stage and also three different forms, rod, spheroid and ring bodies.

The tropical pirosome deviates from the organism found in Tsutsugamushi disease in its typical protozoon staining reaction, and also in

that it does not infect man and cannot be transmitted experimentally to monkeys. The blood parasite of the mole, *Grahamella protista,* Wolbach's (1914) organism in Rocky Mountain tick fever, and *Bartonia bacilliformis* described by Strong and others (1915) in oroya fever, are also more or less closely related but are undoubtedly specifically distinct. I consider the organism in Tsutsugamushi disease as a hitherto undescribed species, and at the suggestion of Dr. Henry B. Ward designate it as *Theileria tsutsugamushi,* n. sp. I am inclined to believe that further study will justify the inclusion of this species in a new genus clearly distinct from that in which it is placed here.

THE PROBABLE LIFE CYCLE OF THE ORGANISM

Since it has not been possible to cultivate the organism, the following successive changes in preparations is the only method for studying its life cycle. For this purpose, all the varieties of granular bodies I have observed were faithfully sketched from preparations (Plate XI):

(a) In Lymphoid Cells: The granular bodies in their smallest form (rod-shaped) are at first chiefly localized in one part of the cytoplasm (Figs. 2a, 3a, 4a). As they increase in size, they become more evenly distributed in the cell body (Figs. 5a, 6a, 7a, 8a) and some show dumb-bell, spheroid, or even ring-shapes. Cells containing large bodies, (Figs. 9a, 10a, 11a, 12a, 13a) gradually disintegrate, setting these bodies free (Figs. 14a, 15a, 16a). At the same time, the chromatin portion of the larger body becomes granular in structure and finally breaks up into minute comma-shaped granules (Fig. 1).

(b) In Red Cells: The changes are similar. A group of minute granular bodies (Figs. 1b) gradually breaks up as they grow into enlarged rods (Figs. 2b, 3b) which continue to increase in size (Fig. 4b) until they become ring-shaped and finally dissociate themselves into minute granular bodies.

(c) In the Plasma: Arranging the free granular bodies according to sizes one would naturally take the minute granular form (Fig. 1) as the starting point, successively followed by the comma or rod form (Figs. 2, 3) and by the spheroid (Figs. 4, 5, 6, 7, 10) or ring-shaped, malaria-like form (Figs. 4 to 9). They may then assume an amoeboid appearance (Figs. 11 to 17) and elaborate minute granules within themselves. These may early break up into minute comma-shaped bodies (Fig. 1) or may form Koch's free "Plasmakugeln" (Figs. 19 to 22) and finally dissolve into comma or rod bodies (Fig. 2).

It is worth noting that in the above interpretation, the stages in the life cycle of my organism correspond very closely to those in the life cycle of *Theileria parva,* as worked out by Gonder.

From the above data the life cycle of the organism of Tsutsugamushi disease may be constructed as follows:

The rod, spheroid or ring-shaped bodies found intracellularly in lymphoid and red blood cells, represent an agamic generation. In progamic and gametic generations the organism is free in the blood plasma, assuming rod or ring shapes (Figs. 2-9), but forms shown in figures 4′, 5′, 6′, 7′, 10 are agamic. In the gametic generation it is transformed into an ameboid body (Figs. 11-17). During the metagamic generation the ameboid body comes to assume an oocyst-like appearance on account of the development of numerous small granules within it (Figs. 16-18). The oocystoid body then breaks down and sets free the granular inclusions, namely the metagametes. The metagametes (sporozoits) are the smallest units of the organism.

Theileria parva is found in the intestine or in the salivary gland of the tick during the metagamic generation. In my organism, however, this generation is also seen in the blood of the patient. In Tsutsugamushi disease, therefore, the mite may not be the necessary intermediate host. In this connection the possibility of infection with this disease by injection of infected blood is of much interest.

It is known that pirosomes multiply by fission. Arguing from analogy then, it is probable that the organism of Tsutsugamushi disease may also, under certain conditions, reproduce in a similar manner. As a matter of fact this organism often assumes a dumb-bell shape suggesting transverse division. If this crosswise division is really a process of reproduction, it should be counted as one of the peculiarities of this organism. The possibility undoubtedly exists that the larger type of dumb-bell shaped bodies appearing in the gametic generation may represent copulation of male and female elements (Pl. XI, Figs. 14, 15, 16, 17), instead of division. It is not possible to come to a definite conclusion on this point at present. The organism of Tsutsugamushi disease differs from Gonder's *T. parva* by its minute comma-shaped true sporozoit.

SUMMARY

1. Minute bodies, described as "rod," "spheroid" and "ring-shaped," have been found in the lymphocytes of lymph nodes and in mononuclear endothelial phagocytes of the spleen and lymph nodes, and in the region of the bite in patients suffering from Tsutsugamushi disease. They also occur free in the blood plasma, and in severe cases in the red cells.

2. The disease has been transmitted experimentally to monkeys, guinea-pigs, rabbits and calves. Bodies similar in appearance and distribution to those found in human cases, have been demonstrated in experimentally infected animals.

3. These bodies, on account of the difficulty of differentiating their cytoplasmic and chromatic elements, resemble bacteria, but no evidence of their bacterial nature has been obtained from cultural or animal experiments.

4. Cultural experiments have proved wholly negative.

5. Microscopically, these bodies are found to possess definite morphology, with parts comparable to the nucleus and cytoplasm of a cell. Moreover, the three principal types in which they appear (rod, spheroid and ring shapes), merge into each other. From these facts, it is concluded that the organism in question is a protozoon. Bodies of different sizes represent different stages in its life cycle.

6. Biologically, this protozoon is to be considered as a new species, resembling but showing differences from *Bartonella bacilliformis* and *Theileria parva*. The parasite to which it appears most closely allied is *Theileria parva.*

7. The organism found in Tsutsugamushi disease, I have designated tentatively as *Theileria tsutsugamushi,* spec. nov.

Here, I wish to express my sincere thanks to Professor A. Fujinami for his continued encouragement and help, and to Mr. Kuhara of Osaka for his generous aid in support of our work. A great deal of credit is also due to the assistants in my laboratory who have participated in the investigations.

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Explanation of Figures

PLATE IX

Drawn from smear preparations with camera lucida and Zeiss 1/12 apo. obj. and comp. oc. 6. Stained by the Giemsa method (Prowazek's trachoma granule stain).

A. Ring-shaped body. *B*. Spheroid body. *C*. Rod-shaped body. *a*. Small ring, and coupled rod and spheroid bodies. *a'*. Small ring-shaped body showing chromatic spot (resembling malaria ring form). *b*. Small spheroid body. *c*. Small rod shaped body.

Fig. 1.—Minute bodies embedded in large mononuclear endothelial cell.

Fig. 2.—Same in small mononuclear lymphoid cell, showing coupled rod and comma-shaped bodies.

Figs. 3, 4, 5.—Similar to Figure 2.

(The plates which are included here through the courtesy of the author are reprinted from the Japanese originals. There they bore the numbers I, II, and III which to fit the series in The Journal have been changed to IX, X, and XI.—Editor.)

PLATE X

Drawn from a smear preparation taken from patient's blood (First published in 1906, Hokuetsu Igakkai Zasshi, No. 156). Drawn from smear made from patient's blood; magnification and staining as in Plate IX.

Fig. 6.—*A,* large, *a,* small ring-shaped body. *B,* large, *b,* small spheroid body. *C,* large, *c,* small rod-shaped body.

Fig. 7.—Large and small rod-shaped bodies embedded in red blood cell.

Figs. 8, 9.—Large rod body, showing three chromatic spots.

Figs. 10, 11.—Small rod and ring-shaped bodies.

Figs. 12, 13.—Ring bodies resembling malaria-rings.

Fig. 14.—Large rod body.

Figs. 15-17.—Large gametocytes connected together by thin thread.

Figs. 18-31.—Smear from bite wound.

Fig. 18.—Rod bodies (*a*) showing achromatic area around chromatin substance. Also another small body with different morphology.

Figs. 19-21.—Rod bodies showing indistinct chromatin substance.

Figs. 22, 24, 26.—Same as figure 18a.

Fig. 24b.—Rod body with two chromatic spots.

Fig. 23.—Same with three achromatic spots.

Fig. 25.—Body with very striking achromatic area.

Figs. 27, 28, 31.—Two coupled gametocytes.

Figs. 29, 30.—Gametocytes; large spheroid body, same as fig. 6 B.

PLATE XI

Diagram of supposed life cycle of *Theileria tsutsugamushi.*

Figs. 1-22.—Free intercellular stage.

Fig. 1.—Sporozoites.

Fig. 2.—Rod-shaped bodies.

Fig. 3.—Elongated rod bodies.

Fig. 4.—Minute spheroid bodies.

Fig. 5.—Same as figure 3, but with an accessory small chromatic spot.

Fig. 6.—Enlarged spheroid bodies (macrogametocytes).

Figs. 7, 8, 9.—Same, like malaria ring body.

Figs. 4, 5, 6, 7, 10.—Spheroid bodies, resembling diplococci.

Figs. 11, 12.—Ring bodies assuming amoeboid appearance.

Fig. 13.—Same, enlarged form.

Fig. 14.—Large bodies coupled together.

Figs. 15, 16.—Large rod bodies, resembling trypanosomes. Note the two nuclei of different sizes and finely granulated protoplasm.

Fig. 17.—Large spheroid bodies, showing fine granulation (oöcyst formation).

Fig. 18.—Same, showing chromatic spot with achromatic ring.

Fig. 19.—Minute granulated body (Koch's free Plasmakugel).

Figs. 20, 21, 22.—Same, containing large granules.

Figs. 1a-16a.—Intracellular stages passed in lymphoid cells.

Fig. 1a.—Spheroid body embedded in mononuclear cell of lymph gland.

Fig. 2a.—Group of minute comma or rod-shaped bodies in mononuclear cell of blood.

Fig. 3a.—Same, showing grouping in mononuclear cell of lymph gland.

Figs. 4a-5a.—Same, showing less localized condition of the bodies.

Figs. 6a-7a.—Same, showing no localization.

Fig. 8a.—Rod and minute ring bodies in splenic cell.

Fig. 9a.—Same, in small round cell of lymph gland.

Fig. 10a.—Same as Plate IX, figure 1.

Fig. 11a-12a.—Rod, spheroid and ring bodies in large mononuclear cell of lymph gland. Fig. 12a also shows cell containing remnants of two rod cells.

Fig. 13a.—Similar to 11a.

Fig. 14a-15a.—Free large ring-shaped bodies identical with intracellular forms.

Fig. 16a.—Free ring-shaped bodies breaking down into minute granular bodies.

Fig. 1b-5b.—Intracellular stage in red blood cells.

Fig. 1b.—Group of minute comma-shaped bodies in red blood cell.

Fig. 2b.—Same, comma- or rod-shaped bodies slightly enlarged.

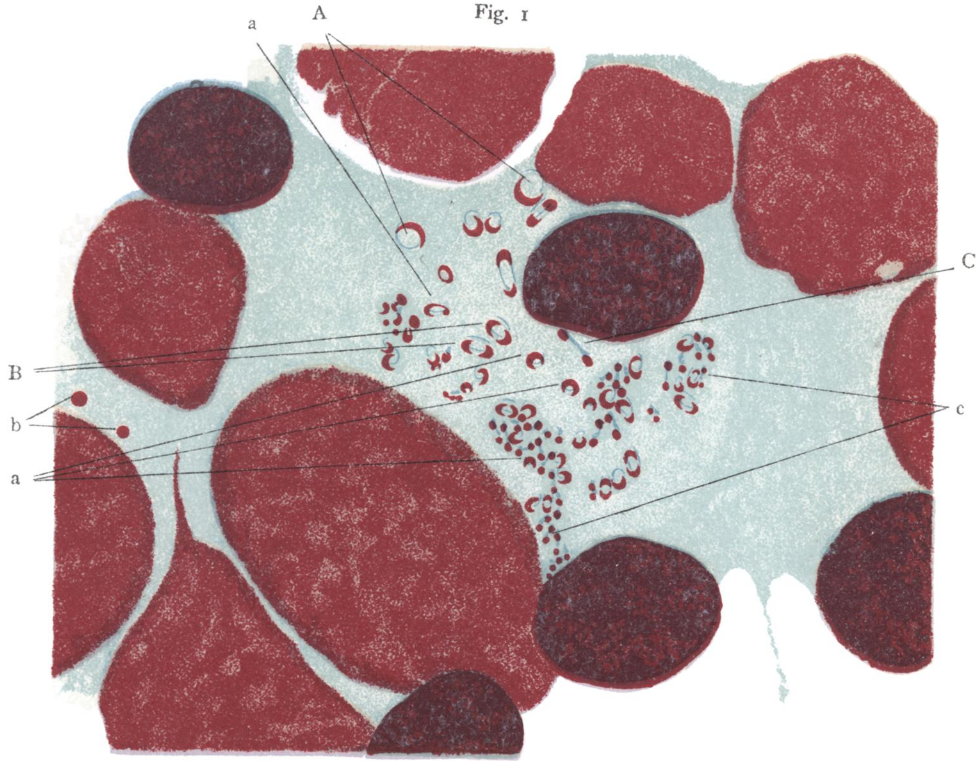
Fig. 3b.—Same, showing further enlargement of comma- or rod-shaped bodies and a few large rod-shaped bodies.

Fig. 4b.—Two large rod-shaped bodies adhering to surface of red cell.

Fig. 5b.—Ring-shaped body, and body showing formation of minute granulation.

Plate I

Fig. 1



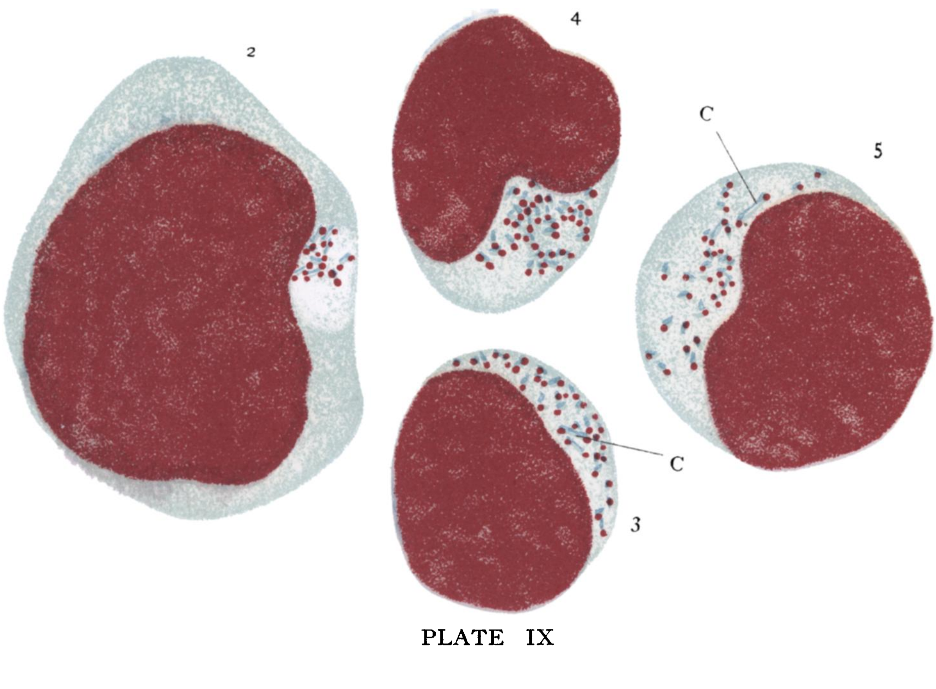


PLATE IX

Plate II

Fig. 6

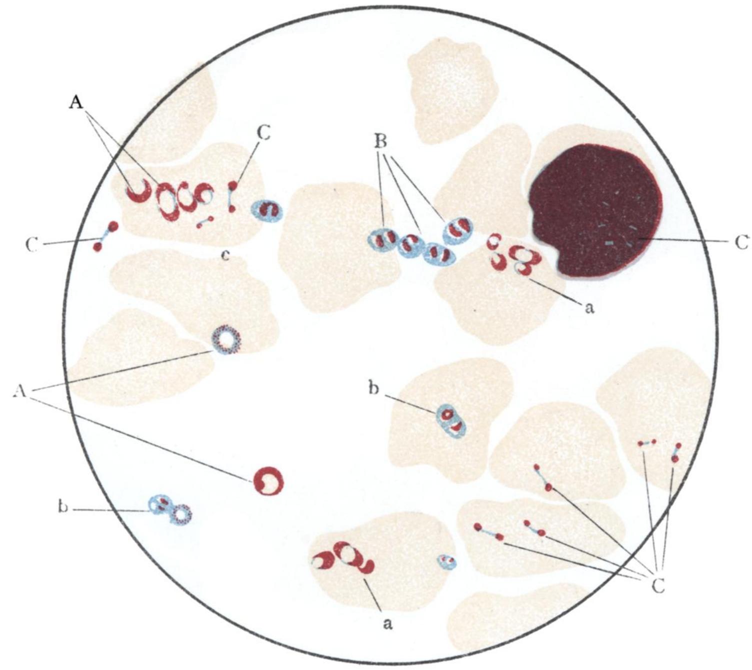


Fig. 7

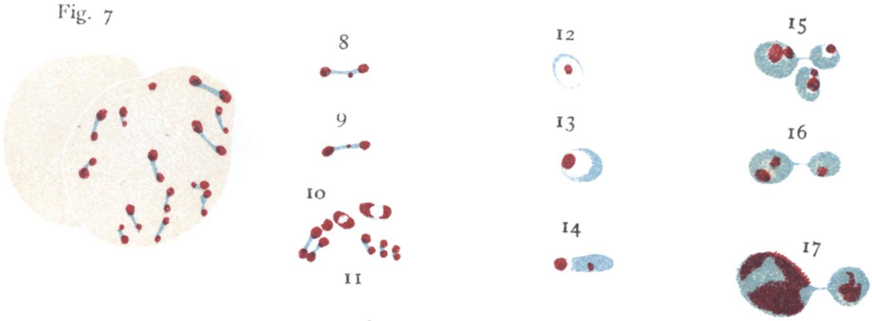


Fig. 18

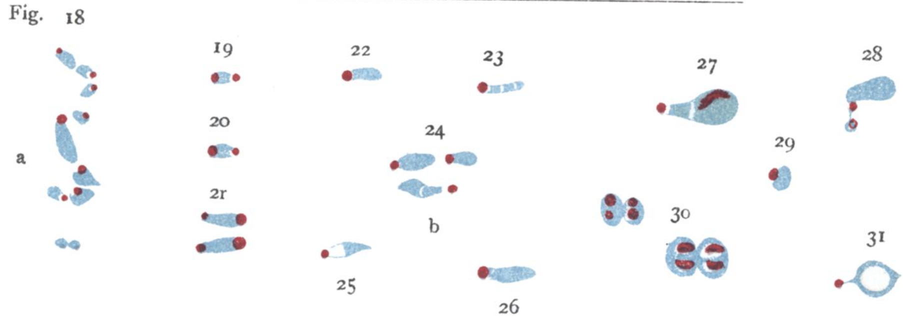
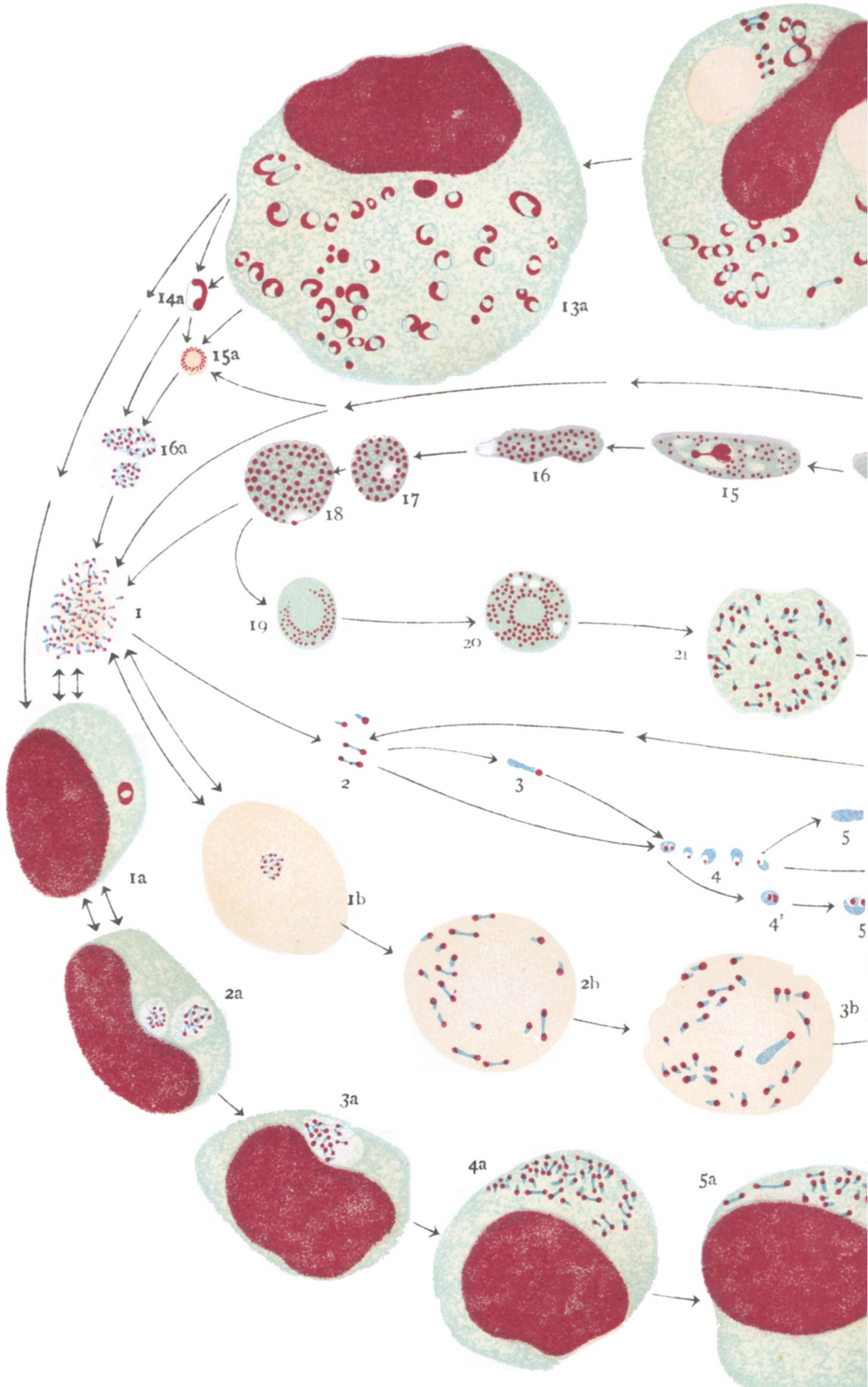


Plate III



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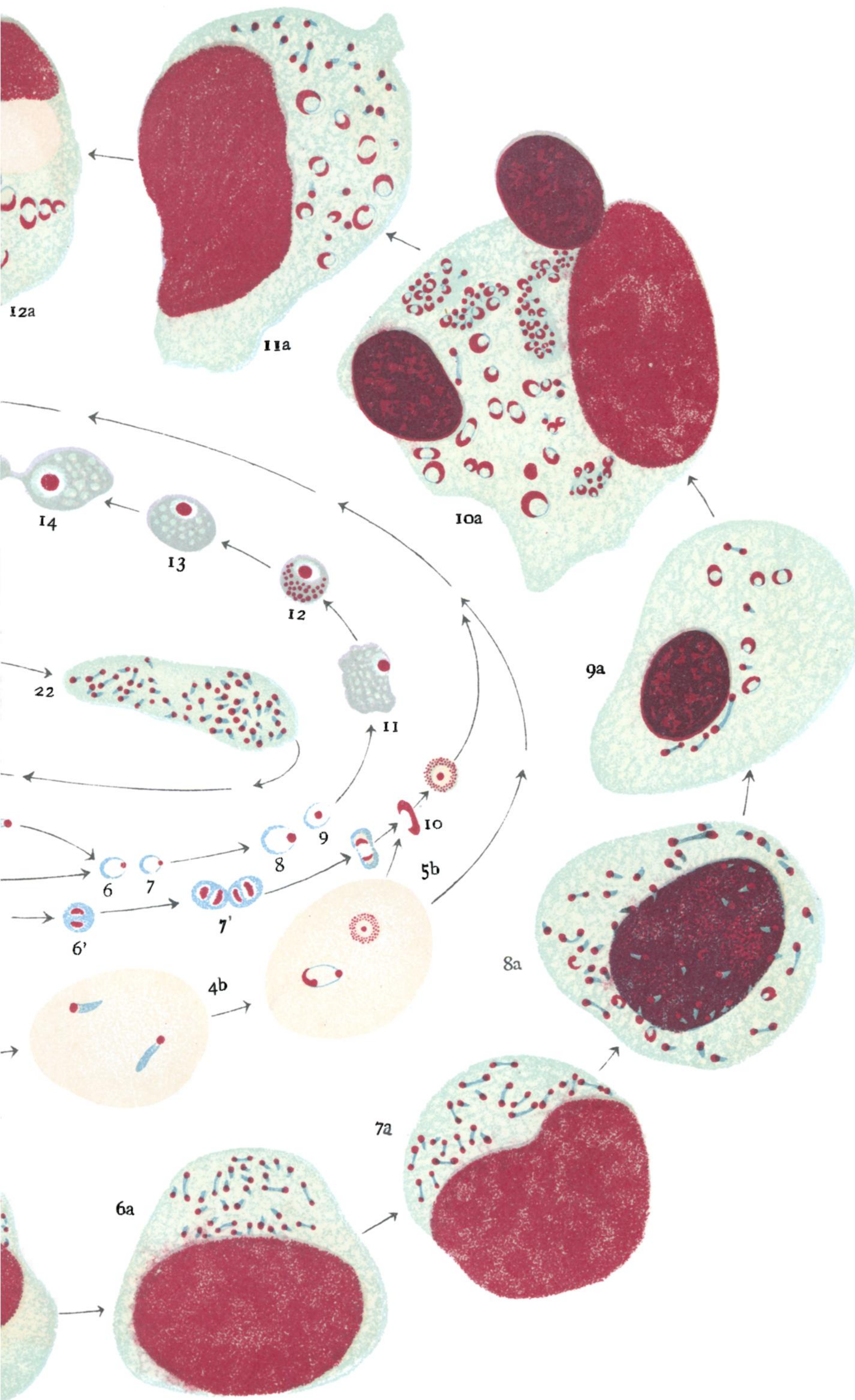

E XI